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PURIFICATION AND CHARACTERIZATION OF MUSCLE PROTEINS FROM *APLYSIA CALIFORNICA*

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Abstract

We report the purification and characterization of the major contractile proteins from body wall muscle of the marine mollusc *Aplysia californica,* and have found that they are quite similar to those of other molluscs. Myosin from this animal consists of heavy chains and at least two different classes of light chains; like the enzymatic activity of other molluscan myosins, its ATPase activity is regulated by Ca^{++}. Actin can be prepared from thin filaments by procedures that minimize denaturation of the protein. *Aplysia* tropomyosin consists of two subunits with apparent molecular weights of 33,000 and 37,000. Paramyosin migrates as a polypeptide of molecular weight 100,000. A description of the procedures for purifying these proteins from *Aplysia* may be useful to neurobiologists who are now using this animal extensively.

Introduction

Invertebrate muscle proteins have been intensively studied because of the insight they provide into the mechanisms that regulate muscle contraction. Scallop muscle is a particularly suitable source of material because it is available in large amounts as a homogeneous tissue (Szent-Györgyi *et al.,* 1973). Studies of molluscan muscle show that contraction is triggered by binding of Ca^{++} to myosin in thick filaments, rather than to troponin in thin filaments as in vertebrate muscle (Lehman and Szent-Györgyi, 1975). These studies have also demonstrated that a distinctive component of invertebrate muscle is paramyosin, a protein whose function is not yet clear, but which is thought to serve a structural role in thick filaments (Szent-Györgyi *et al.,* 1971).

During the past decade, contractile proteins have also received considerable attention from cell biologists interested in the mechanism of motility in non-muscle cells (see, for example, Taylor and Condeelis, 1979; Bray and Gilbert, 1981; Brinkley, 1982). Our interest concerns the possible participation of actin and myosin in fast axoplasmic transport, a form of intracellular motility in which synaptic vesicles and other membranous organelles are moved along the axon of a nerve cell to its terminals (Schwartz, 1979; Grafstein and Forman, 1980). Our experimental strategy is to microinject anti-actin antibodies and other binding proteins into giant neurons of the marine mollusc, *Aplysia* (Goldberg *et al.,* 1980; Goldberg, 1982). To obtain sufficient quantities of the contractile proteins to raise antibodies, we isolated actin, myosin, tropomyosin, and paramyosin from *Aplysia* muscle. Here we report the purification

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Abbreviations: HMM = heavy meromyosin; LMM = light meromyosin; SDS = sodium dodecyl sulfate.

and characterization of these proteins. Biochemical studies of *Aplysia* actin are of special interest because the antibody raised against the purified protein has unusual specificity: it cross-reacts only with cytoplasmic actin but not with sarcomeric actin from vertebrates (Lubit and Schwartz, 1980; Hall *et al.*, 1981; Lubit and Schwartz, 1983).

Materials and Methods

Muscle from body wall, buccal mass, and heart was obtained from *Aplysia californica* purchased from Pacific Bio-Marine, Venice, California, or raised at the *Aplysia* Mariculture Facility at the Marine Biological Laboratory, Woods Hole, Massachusetts. Animals were extended with their ventral surface up, pinned rostrally and caudally, and opened with an incision along the entire length of the foot. The viscera were removed, and the flaps of body wall stretched and pinned laterally until taut. The buccal mass was removed, cleaned of the buccal ganglion and attached nerves, and the red buccal muscle dissected from the radula. The heart was freed from the pericardium. Muscle from the body wall was most efficiently obtained by separating it as a single strip from the skin using a sharp scalpel. Dissection began laterally and proceeded toward the midline. As the muscle was freed from the skin it contracted into a thick band on each side of the animal. These bands were cut at their ends and peeled away from the carcass.

Muscle tissue was rinsed briefly at 0°C in 40 m*M* NaCl, 1 m*M* $MgCl_2$, 0.1 m*M* EDTA, 5 m*M* sodium phosphate (pH 7.0), and was either used immediately or stored at −20°C in 50% glycerol. Glycerinated muscle was stable for six months, after which significant proteolysis of contractile proteins (especially myosin) occurred, and recovery of purified proteins was low.

Myosin and meromyosins

Myosin was prepared by ammonium sulfate fractionation of actomyosin as described by Chantler and Szent-Györgyi (1978), and was purified further by gel filtration on a column (1.5 × 85 cm) of Sepharose 4B-CL (Pharmacia, Piscataway, New Jersey) in 0.6 *M* NaCl, 0.2 m*M* dithiothreitol, 1 m*M* sodium pyrophosphate, and 5 m*M* sodium phosphate (pH 7.0). It was stored either at 4°C as a suspension in 65% ammonium sulfate, or at −20°C in 50% glycerol. Myosin from rabbit skeletal muscle was prepared by the procedure of Mommaerts and Parrish (1951).

To prepare heavy and light meromyosins, myosin from body wall muscle (2–25 mg/ml) was digested with 0.2% by weight of trypsin (Type DCC, Miles Laboratories, Elkhart, Indiana) at 25°C in 0.5 *M* KCl and 30 m*M* Tris-HCl (pH 8.2) (Balint *et al.*, 1975); 1 m*M* $MgCl_2$ was also present (Craig *et al.*, 1980). Digestion was terminated by the addition of soybean trypsin inhibitor (Type I-S, Sigma Chemical Co., St Louis, Missouri) equal to twice the weight of trypsin. The reaction mixture was dialyzed against 20 m*M* NaCl, 1 mm $MgCl_2$, 0.1 m*M* EGTA, 0.1 m*M* dithiothreitol, 5 m*M* sodium phosphate (pH 7.0), and was centrifuged at 30,000 × *g* for 30 min to remove the precipitate of light meromyosin (LMM) and undigested myosin. The supernatant contained heavy meromyosin (HMM). The precipitate was washed several times with 5 m*M* sodium phosphate (pH 6.5), and LMM purified from it by ethanol fractionation (Lowey *et al.*, 1969).

Thin filaments and actin

Thin filaments were prepared by extracting myofibrils at pH 6 (Szent-Györgyi *et al.*, 1971). Actin was purified from thin filaments by two methods: (1) application

of filaments to a column (2.5 × 30 cm) of DEAE-cellulose, followed by elution with a KCl gradient using the conditions of Gordon *et al.* (1976); actin-containing fractions were combined, and the protein polymerized and sedimented as described (Gordon *et al.*, 1976); (2) extraction of an acetone powder (Szent-Györgyi, 1951) of thin filaments with 0.5 m*M* 2-mercaptoethanol (pH 6–6.5) for 30 min at 25°C; after addition of 10 m*M* $MgCl_2$, the extract was kept at 25°C for another 1.5 h, and F-actin was collected by centrifugation at 100,000 × *g* for 3 h.

Actins from rabbit skeletal muscle and chicken gizzard were prepared by the method of Spudich and Watt (1971).

To examine thin filaments by electron microscopy, 0.1 mg/ml samples were applied to Formvar-carbon-coated grids, negatively stained with 1% uranyl acetate, and viewed in a Philips 301 electron microscope.

Tropomyosin and paramyosin

Tropomyosin was obtained during chromatography of thin filaments on DEAE cellulose (see above, method 1). Fractions containing tropomyosin were made 10 m*M* in $MgCl_2$ and centrifuged at 100,000 × *g* for 5 h to remove actin. Tropomyosin was also extracted along with actin from the acetone powder of thin filaments (see above, method 2). The supernatant remaining after sedimentation of F-actin was enriched in tropomyosin.

Paramyosin was isolated by the procedure of Szent-Györgyi *et al.* (1971), and purified further by addition of ice-cold acetone and extraction of the dried acetone powder at 25°C with 0.1 *M* NaCl and 5 m*M* sodium phosphate (pH 7.4). The extract was again precipitated with acetone and extracted once more with the same buffer.

Immunological procedures

To raise antibodies against intact myosin (heavy and light chains), the purified protein from body wall muscle was electrophoresed on 5% polyacrylamide gels in the absence of sodium dodecyl sulfate (SDS). The gels were stained with Coomassie Blue, and the region of the gel containing myosin was cut out, lyophilized, and powdered. The powder was suspended in 0.85% NaCl using a Teflon-glass homogenizer and was then emulsified with one-half volume of Freund's complete adjuvant (Gibco, Grand Island, New York). New Zealand white rabbits were injected intramuscularly in their hind legs with emulsion containing 20–25 μg of myosin once per week for four weeks, and were boosted monthly thereafter with the same amount of immunogen. Rabbits were bled weekly beginning one week after the initial series of injections. Immunoglobulin was prepared from antisera by fractionation with ammonium sulfate at 50% saturation.

To raise antibodies against heavy and light chains separately, the purified protein was electrophoresed in polyacrylamide gels containing SDS, and the regions of the stained gels containing the subunits of myosin were excised and prepared as above. Light chains of both high and low molecular weight were used together for immunization.

Double immunodiffusion on Ouchterlony plates was carried out using glass microscope slides covered with 1% agarose (type I, Sigma) in 20 m*M* sodium pyrophosphate (pH 8.6). Plates were kept in a moist chamber at 25°C for 1–3 days, and were then washed, dried, and stained with Coomassie Blue.

Analytical procedures

Electrophoresis on polyacrylamide gels was carried out with SDS by the method of Laemmli (1970) or of Weber and Osborne (1969). Gradient slab gels of 4–30%

polyacrylamide were prepared using the gel and electrode buffers of Weber and Osborne (1969).

ATPase was assayed at 25°C by measuring the amount of P_i released using the method of Taussky and Schorr (1953). In some experiments, ATP hydrolysis was calculated from the rate of proton release using a pH-stat with an automatic titrator (Radiometer, Copenhagen).

Protein was assayed colorimetrically either by the method of Bradford (1976) or of Lowry *et al.* (1951), using bovine gamma globulin as standard.

Results

Myosin

Myosin from body wall muscle of *Aplysia* was prepared using Chantler and Szent-Györgyi's (1978) procedure for isolating other invertebrate myosins (Fig. 1). Extraction

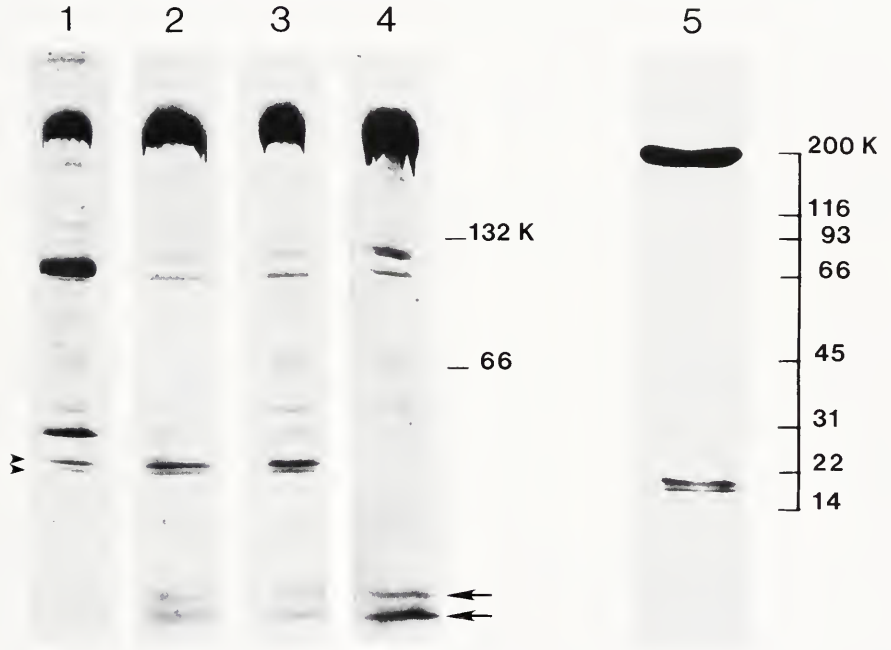

FIGURE 1. Analysis of proteins obtained during the purification of myosin from *Aplysia* body wall muscle. Samples were analyzed by electrophoresis in SDS on gradient slab gels of 4–30% polyacrylamide (lanes 1–4) or on 9% polyacrylamide slab gels (lane 5). The molecular weight markers for lanes 1–4 are monomeric (66,000) and dimeric (132,000) bovine serum albumin; the markers for lane 5 are rabbit myosin (200,000), beta-galactosidase (116,000), phosphorylase b (93,000), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (22,000), and lysozyme (14,000).

Lane 1: Actomyosin obtained by extraction of myofibrils with 0.6 *M* NaCl and 5 m*M* ATP (10 μg protein). The arrowheads indicate the positions of the two subunits of tropomyosin. Lane 2: Supernatant obtained after fractionation of actomyosin with ammonium sulfate at 40% saturation (20 μg). Lane 3: Myosin precipitated by further fractionation with ammonium sulfate to 65% saturation (10 μg). Lane 4: Myosin after precipitation at low ionic-strength and washing (22 μg). The arrows indicate the positions of the two classes of myosin light chains. Lane 5: Peak fractions after gel filtration of myosin on Sepharose 4B-CL (20 μg). Two bands are visible in the region between the 22,000 and 14,000 molecular weight markers, but the lower band is an artifact of electrophoresis and was present even in gel lanes that did not contain protein.

of myofibrils with 0.6 *M* NaCl and 5 m*M* ATP at pH 7 solubilizes myosin, paramyosin, actin, and tropomyosin (lane 1). Addition of ammonium sulfate to 40% saturation removes most of the paramyosin and actin (lane 2). Further addition of ammonium sulfate to 65% saturation precipitates myosin, which can be solubilized again (lane 3) and then freed of tropomyosin by precipitation at low ionic strength and washing. The final preparation (lane 4) consists principally of myosin, but still contains small amounts of other proteins. These contaminants could be almost completely removed by gel filtration on a column of Sepharose 4B-CL (Fig. 1, lane 5). In six independent preparations, recovery of gel-filtered myosin ranged from 2.4–3.4 mg per gram wet-weight of muscle.

Aplysia myosin, like other vertebrate and invertebrate myosins, consists of heavy and light chains. On SDS-polyacrylamide gels (Laemmli, 1970), the heavy chain migrates with an apparent molecular weight of 200,000 and the light chains appear as a single, diffuse band with an apparent molecular weight of 19,000 (Fig. 1, lane 5). In gradient gels, the light chains can be resolved into two components with molecular weights of 18,500 and 19,000 (Fig. 1, lane 4). The 18,500 molecular weight component stains more darkly with Coomassie Blue, and presumably is present in greater molar amounts.

To investigate whether *Aplysia* myosin is immunologically related to vertebrate myosins, we raised antisera in rabbits against the intact *Aplysia* protein, as well as against its heavy and light chains separately. Although they all gave a single, sharp precipitin line with both purified *Aplysia* myosin and a crude extract of body wall muscle, none of these antisera were found to cross-react during double-immunodiffusion with myosin from rabbit skeletal muscle (Fig. 2). The antisera also did not cross-react with actomyosin from chicken gizzard, or with actin from body wall muscle of *Aplysia,* rabbit skeletal muscle, or chicken gizzard.

ATPase activity of myosin and meromyosins

The ATPase activity of myosin purified from *Aplysia* body wall muscle is inhibited by Mg^{++} and activated by Ca^{++} and K^{+}/EDTA (Table I), features that are typical of myosins from both vertebrate and invertebrate sources. In addition, the ATPase activity of *Aplysia* myosin is stimulated ten-fold by purified actin, but only in the presence of Ca^{++} (Table I). This dependence on Ca^{++} is characteristic of myosins from molluscan species that display thick filament-linked regulation of contraction (Lehman and Szent-Györgyi, 1975).

Heavy and light meromyosins can be prepared from *Aplysia* myosin by a three-minute digestion with trypsin. As expected from studies with meromyosins from rabbit skeletal muscle, the ATPase activity of *Aplysia* myosin resides entirely in the HMM portion of the molecule: in the presence of Ca^{++}, HMM had an activity of 0.49 μmoles P_i/min/mg, compared to LMM with an activity of 0.01 (for assay conditions, see legend to Table I).

Thin filaments and actin

Thin filaments can be prepared from body wall muscle of *Aplysia* using the method of Szent-Györgyi *et al.* (1971), in which myofibrils are extracted at pH 6 with ATP and EDTA. Electron microscopic examination reveals filaments with a diameter of 6 nm and a range of different lengths (Fig. 3). We did not observe any dense bodies attached to the ends of filaments (Szent-Györgyi *et al.,* (1971). Actin, with an apparent molecular weight of 43,000, is the major protein in this preparation, although other proteins are also present (Fig. 4, lane 1). Some of these proteins,

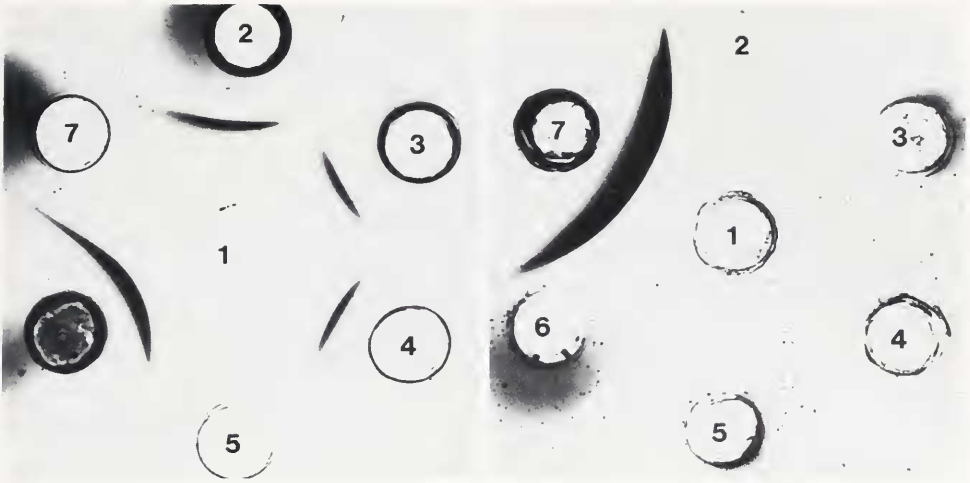

FIGURE 2. Double immunodiffusion using antiserum raised against intact myosin from *Aplysia* body wall muscle. Ouchterlony plates were washed, dried, and stained with Coomassie Blue. The wells contained:

Left: (1) immunoglobulin fraction of antiserum (2 mg/ml), (2–5) myosin purified from body wall muscle (0.2–1.4 mg/ml), (6) crude extract of body wall muscle in 0.6 *M* NaCl (8 mg/ml), and (7) rabbit skeletal myosin (1.4 mg/ml).

Right: (1) immunoglobulin fraction of antiserum (2 mg/ml), (2) rabbit skeletal actin (3.8 mg/ml), (3) actin from body wall muscle (1.5 mg/ml), (4) actin from chicken gizzard smooth muscle (1.5 mg/ml), (5) actomyosin extracted from chicken gizzard myofibrils in 0.6 *M* NaCl (8 mg/ml), (6) rabbit skeletal myosin (1.4 mg/ml), and (7) extract of body wall muscle in 0.6 *M* NaCl (8 mg/ml).

including tropomyosin, sediment with actin after centrifugation of filaments at 100,000 $\times$ *g* for 3 h (not shown). Typically, the overall yield of protein in thin filament preparations was 1 mg per gram wet-weight of muscle. Thin filaments could also be prepared from buccal and heart muscle (not shown).

TABLE I

ATPase Activity of Aplysia *myosin*

Myosin alone[1]	Activity[2]
Ca^{++}	0.35
Mg^{++}	0.02
K^{+}/EDTA	0.37
Effect of actin[3]	
Actin, Ca^{++}	0.22
Actin, EGTA	0.06
No actin, Ca^{++}	0.02

[1] The assay mixtures contained 5 m*M* $CaCl_2$/0.6 *M* NaCl; 5 m*M* $MgCl_2$/0.1 m*M* EGTA/0.6 *M* NaCl; or 2 m*M* EDTA/0.6 *M* KCl.

[2] Assays were conducted at pH 7.5 in 10 m*M* imidazole-HCl buffer containing 1 m*M* ATP. ATPase activity is expressed as μmoles P_i/min/mg protein and was determined after a 10 min incubation at 25°C; the rate of P_i release during this time was found to be constant. Values are typical, and represent the average of duplicate determinations. Similar values were obtained with at least two and as many as four independent preparations of *Aplysia* myosin. Assays were carried out over a period of six months with little variation.

[3] The assay mixture contained 0.35 m*M* $CaCl_2$/0.35 m*M* $MgCl_2$/30 m*M* NaCl; or 0.1 m*M* EGTA/0.35 m*M* $MgCl_2$/30 m*M* NaCl. Rabbit F-actin was added at a ratio of 0.5–0.7 mg/mg of myosin.

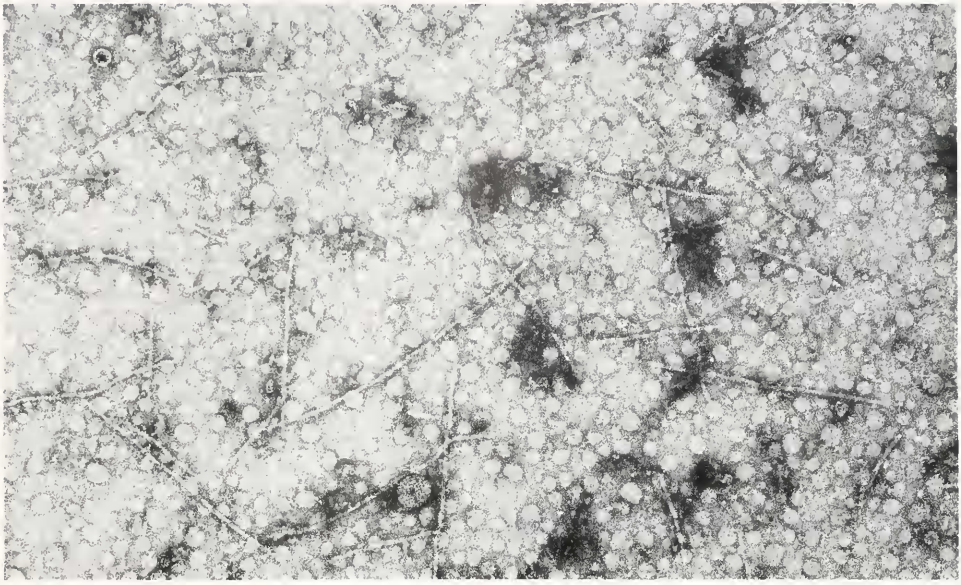

FIGURE 3. Electron micrograph of thin filaments from *Aplysia* body wall muscle. Magnification 77,000×. The filaments, negatively stained with 1% uranyl acetate, are seen against a background of droplets of stain.

We purified actin from *Aplysia* thin filaments by two methods. The first, chromatography on DEAE-cellulose (Gordon *et al.*, 1976), seemed advantageous because we found that *Aplysia* actin is denatured during the successive cycles of polymerization and depolymerization that are required in other purification procedures (Spudich and Watt, 1971). When a gradient of KCl was applied to the ion-exchange resin, protein was eluted in several peaks (Fig. 5). Electrophoretic analysis of these fractions showed that actin was eluted between 0.22 and 0.26 *M* KCl (Fig. 4, lanes 2–4). After the actin-containing fractions were combined and the protein polymerized by addition of 2 m*M* $MgCl_2$, essentially pure F-actin free of tropomyosin could be collected by centrifugation at 100,000 × *g* for 5 h (not shown). Typically, 0.5 mg of actin could be obtained per mg of thin filaments. Tropomyosin was eluted at higher salt concentrations (see below).

In the second method, actin was extracted from an acetone powder of thin filaments and then polymerized by the addition of $MgCl_2$. The F-actin collected by centrifugation was still contaminated with a small amount of tropomyosin (Fig. 4, lane 7).

Tropomyosin

Fractions eluting between 0.28 and 0.30 *M* KCl during chromatography of thin filaments on DEAE-cellulose contained tropomyosin contaminated by only traces of actin (Fig. 4, lanes 5, 6). Addition of $MgCl_2$ and centrifugation at high speed removed most of the actin (not shown). 0.2 mg of purified tropomyosin was obtained per mg of thin filaments. Relatively pure tropomyosin also could be obtained in the supernatant that remained after collection of F-actin from the extract of an acetone powder of thin filaments (Fig. 6, lane 1). On 5% SDS-polyacrylamide gels, tropomyosin appeared as a single, broad band with a molecular weight of 35,000 (Fig. 6). On 4–30% gradient gels, it could be resolved into two subunits with apparent molecular weights of 33,000 and 37,000 (Fig. 1, lane 1).

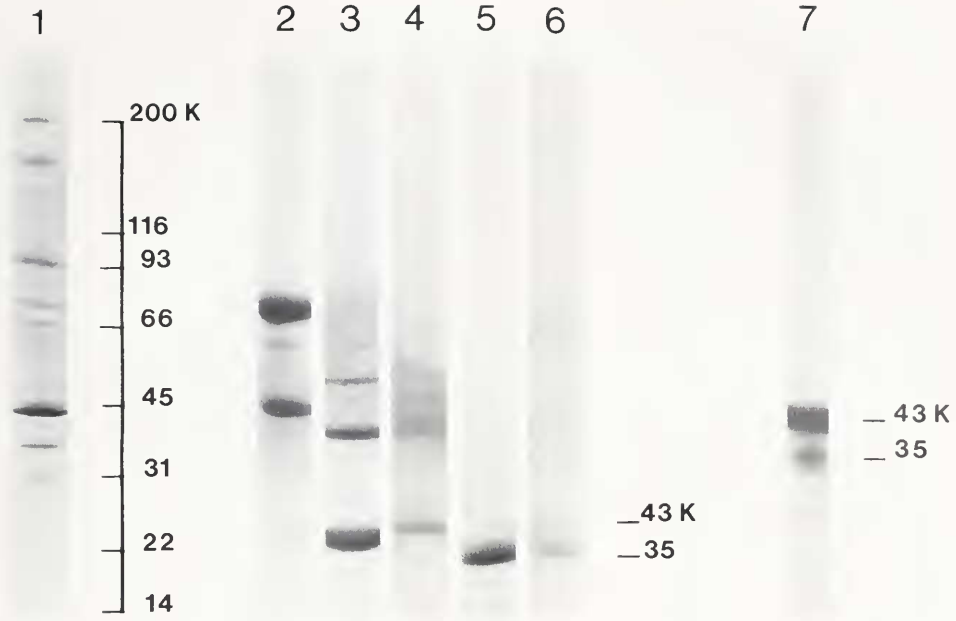

FIGURE 4. Analysis of thin filaments and actin from *Aplysia* body wall muscle. Samples were analyzed by electrophoresis in SDS on gradient slab gels of 4–30% polyacrylamide (lanes 1–6) or on a 5% polyacrylamide tube gel (lane 7). The molecular weight markers for lane 1 are the same as for lane 5 of Figure 1; for lanes 2–7 the markers are actin (43,000) and tropomyosin (35,000) from rabbit skeletal muscle.

Lane 1: thin filaments (15 μg protein), lanes 2–6: fifty μl samples of the fractions eluted during DEAE-cellulose chromatography of thin filaments described in the legend to Figure 5; lane 2, fraction no. 39; lane 3, fraction no. 42; lane 4, fraction no. 46; lane 5, fraction no. 49; lane 6, fraction no. 53; lane 7: actin purified from an acetone powder of thin filaments (20 μg).

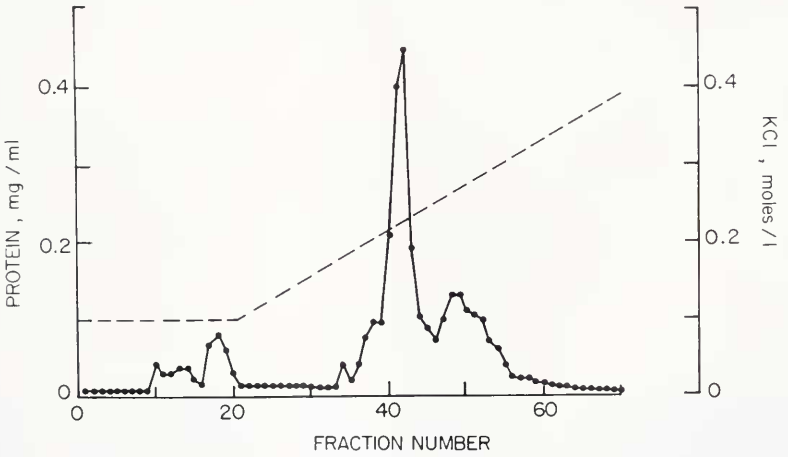

FIGURE 5. Chromatography of thin filaments from *Aplysia* body wall muscle on DEAE-cellulose. The column (2.5 × 30 cm) was equilibrated with 0.1 *M* KCl in Buffer D of Gordon *et al.*, 1976 (10 m*M* imidazole-Cl (pH 7.5), 0.1 m*M* $CaCl_2$, 0.5 m*M* ATP, 0.75 m*M* beta-mercaptoethanol). 25 ml of Buffer G (same as Buffer D, but with 3 m*M* imidazole-Cl) was then applied, followed by 50 mg of thin filaments (0.3 mg/ml), and then 25 ml more of Buffer G. The column was then eluted with 125 ml of 0.1 *M* KCl in Buffer D and then a linear gradient (1 liter) of 0.1 *M* to 0.5 *M* KCl in Buffer D. Fractions of 17 ml were collected; ——, protein, – – –, concentration of KCl.

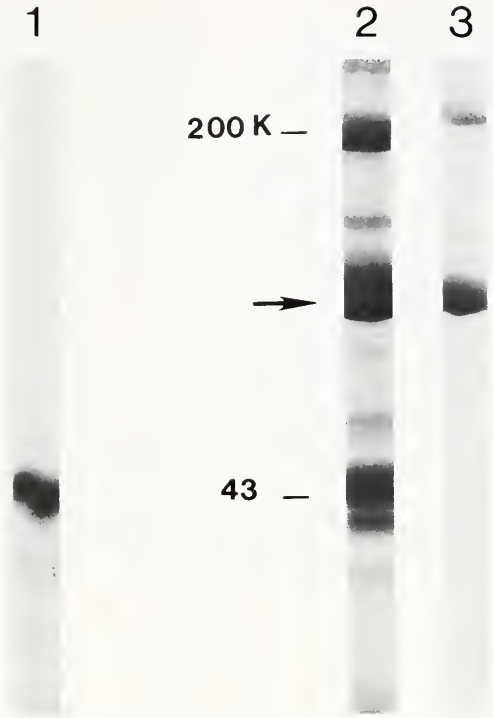

FIGURE 6. Analysis of tropomyosin and paramyosin from *Aplysia* body wall muscle. Samples were analyzed by electrophoresis in SDS on 5% polyacrylamide tube gels. Molecular weight markers for lanes 2 and 3 are myosin heavy chain (M_r 200,000) and actin (M_r 43,000), both from rabbit skeletal muscle.

Lane 1: Tropomyosin purified from an acetone powder of thin filaments [15 μg; the protein comigrated with tropomyosin from rabbit skeletal muscle (M_r 35,000)]; lane 2: paramyosin (arrow) prepared by differential extraction of thick filaments (25 μg); and lane 3: paramyosin purified further by acetone precipitation (15 μg). A small amount of myosin heavy chain remains.

Paramyosin

Paramyosin prepared from body wall muscle by differential extraction of thick filaments (Szent-Györgyi *et al.*, 1971) was contaminated with myosin and actin (Fig. 6, lane 2). After addition of acetone and extraction at pH 7.4 two times, the preparation was highly enriched in paramyosin (Fig. 6, lane 3), which had a molecular weight of 100,000.

DISCUSSION

Myosin

Aplysia myosin, purified from body wall muscle by ammonium sulfate fractionation (Chantler and Szent-Györgyi, 1978) followed by gel filtration, is similar to myosins from other molluscan species. The purified protein consists of heavy chains of molecular weight 200,000 and two classes of light chains with molecular weights of 18,500 and 19,000. Szent-Györgyi *et al.* (1973) have found that scallop myosin also contains heavy chains of molecular weight 200,000 and light chains of molecular weight 18,000. Scallop light chains can be resolved into two components by electrophoresis on urea-polyacrylamide gels. One type of light chain can be selectively removed from myosin

by EDTA and is responsible for conferring Ca^{++}-sensitivity on the actin-activated ATPase activity of the molecule. The other light chain contains cysteine residues and its function is unknown. There are two light chains of each type in a molecule of scallop myosin (Kendrick-Jones *et al.*, 1976).

Although vertebrate skeletal myosin also contains both heavy and light chains, these subunits appear to be functionally and structurally distinct from their molluscan counterparts (Kendrick-Jones *et al.*, 1976). Consistent with these differences, we have found that antibodies raised against *Aplysia* myosin, as well as against its separated heavy and light chains, do not cross-react with the myosins from chicken gizzard or rabbit skeletal muscle.

Contraction of vertebrate skeletal muscle is triggered by binding of Ca^{++} to troponin-containing thin filaments. In contrast, Lehman and Szent-Györgyi (1975) have shown that binding of Ca^{++} to myosin in thick filaments initiates contraction in molluscan muscle. One biochemical correlate of myosin-linked regulation is the ability of rabbit actin to stimulate the ATPase activity of molluscan myosin in the presence, but not in the absence, of Ca^{++}. By this criterion, *Aplysia* muscle, like that of other molluscs, displays myosin-linked regulation; when Ca^{++} is omitted, the actin-stimulated ATPase activity of *Aplysia* myosin is reduced by 74% (Table I). In the same assay, the ATPase activity of rabbit myosin is slightly greater when Ca^{++} is absent (data not shown).

Thin filaments and actin

Thin filaments can be prepared from body wall muscle of *Aplysia* by extraction of myofibrils with ATP and EDTA. We chose to purify actin from thin filaments, rather than from an acetone powder of myosin-depleted whole muscle (Spudich and Watt, 1971), because we found that the yield of actin using the latter procedure was poor. One reason for the low yield may be that *Aplysia* actin was easily denatured during the polymerization at high ionic strength that is used in the procedure of Spudich and Watt. Purified *Aplysia* actin was prepared by chromatography of thin filaments on DEAE-cellulose (Gordon *et al.*, 1976), or by extraction of an acetone powder of thin filaments. We also have purified denatured *Aplysia* actin by preparative SDS gel electrophoresis (Sherbany and Schwartz, unpub.), and have used it as an immunogen to raise an antiserum that cross-reacts specifically with vertebrate cytoplasmic actin (Lubit and Schwartz, 1980, 1983).

Tropomyosin and paramyosin

Tropomyosin from body wall muscle of *Aplysia,* like vertebrate skeletal tropomyosin, consists of two subunits that migrate on polyacrylamide gels with apparent molecular weights of 33,000 and 37,000. *Aplysia* paramyosin has a molecular weight of about 100,000, and is of considerable interest because it is the major phosphoprotein in muscle. Stimulation of the identified serotonergic giant cerebral neuron or application of serotonin causes an elevation in the synthesis of cAMP within buccal muscle (Weiss *et al.*, 1979), and this, in turn, leads to the increased phosphorylation of paramyosin (Weiss *et al.*, 1981). Application of serotonin also promotes the phosphorylation of paramyosin in other *Aplysia* muscles as well as in nervous tissue (Katz *et al.*, 1983). Work is now in progress to determine whether the paramyosin found in extracts of nervous tissue is a contaminant from the muscular connective tissue sheath of the ganglia or a native constituent of nerve cells.

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